

REMARKS

Claims 16, 20, and 24 have been amended; claim 18 has been cancelled; and new claims 25-29 have been added. Claims 16-17 and 19-29 are currently pending in the application. All pending claims are set forth in Exhibit A with amendments shown (if applicable).

Applicants gratefully acknowledge the 14 February 2003 interview with the Examiner in which all pending rejections in the application were discussed. In particular, the role of the capture ligand in Applicants' invention was explained and differentiated from the use of capture ligands in Babon (cited below).

The specification has been amended to add a paragraph from parent application 09/698,846 that was incorporated by reference into the present application (see page 1, lines 5-8). This paragraph is found on page 9, line 25, to page 10, line 4, of 09/698,846, and has been expressly incorporated by the above amendment because it contains terms used in the claims as noted in the table below.

The specification has also been amended to add a Sequence Listing section to list the sequences set forth on page 49, lines 25-30, and page 60, line 37 of the specification.

The claims have been amended to more clearly define Applicants' invention with respect to the capture ligand and its function. New claims have been added to provide an alternative description of Applicants' invention wherein mobility modifier components of eTag reporters are defined in terms of molecular weight.

Bases for amendments to the claims are as follows:

Claim(s)	Term/Phrase	Basis
16, 25	"M is a non-oligomeric compound"	Page 30, lines 30-31. Claims 8, 13, and 23 of parent application 09/602,586 that has been incorporated by reference.
16, 25	"released from an electrophoretic probe of the set upon digestion of the electrophoretic probe by a nuclease" in reference to the eTag reporter (D,M)-N.	Page 4, lines 19-24 (describes concept of (D,M)-N being release from electrophoretic probe). Page 31, lines 2-6 (states that the methodology of the invention may be implemented with the nucleases used in polymerase chain reaction and Invader technologies). Page 33, Table 3 (rightmost column entitled "e-tag Release" at top lists four (4) exemplary nucleases for releasing eTag reporters)
16, 25	"in an electropherogram" in reference to distinct peaks of eTag reporters separated by electrophoresis.	Page 5, lines 40-42 (figure caption for Fig. 8) Figure 8.

16, 25	"capture ligand specifically binds to a capture agent to exclude undigested electrophoretic probes from the electropherogram"	Claim 1, page 68, lines 21-22 (concept of capture ligand specifically binding to capture agent). Page 24, lines 19-22 (describes the function of a capture ligand). Page 7, lines 1-5, Figure captions for Figs. 26 and 27. Figures 26 and 27 showing data wherein undigested probes are excluded from electropherogram. Figure 3B showing diagrammatically the exclusion of undigested probe.
25	"molecular weight of between 35 and 1500 daltons" in reference to M.	Page 9, lines 27-31, of parent application 09/698,846*.
29	Chemical formula for eTag reporters.	Figure 15.

* Parent application 09/698,846 was incorporated by reference (see page 1, lines 5-8, of the specification), and the indicated passage has been expressly incorporated by the above amendment to the specification.

No new matter has been added by the amendments. Reconsideration is respectfully requested.

Rejection Under 35 U.S.C. 112 Second Paragraph

The Examiner rejected claims 18 and 20 under 35 U.S.C. 112 second paragraph because of the phrase "charge/mass ratio is in the range of from -.001 to 0.5," which the Examiner averred was unclear as how such ratios are defined.

Applicants respectfully disagree with these rejections and submit that one of ordinary skill in the art would understand the meaning of the ratios, particularly in view of the computed examples given in Tables 1 and 2 on pages 21 and 22 of the specification, respectively. However, in order to move the prosecution of the application forward the language at issue has been remove. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. 103

The Examiner rejected claim ^{16-17, 19-21, 28-34} 5 under 35 U.S.C. 103(a) as being unpatentable over Grossman (5,470,705) in view of Babon (5,851,770). The Examiner argues as follows: Grossman discloses a method and compositions for detecting a plurality of polynucleotide sequences, the compositions comprising a plurality of probes each consisting of an oligonucleotide and a polymer chain that gives the probe a distinct electrophoretic mobility. Babon discloses use of a capture ligand, such as biotin, to capture on a solid phase support various hetero- and homoduplexes that may or may not contain mismatched basepairs. Captured duplexes are treated with a mismatch-recognizing nuclease that cleaves the captured sequences at mismatch locations to release fragments which are then analyzed by electrophoresis. The Examiner argues that it would be

obvious to one of ordinary skill to modify the probes of Grossman to include the capture ligands of Babon, thereby obtaining Applicants' invention. One of ordinary skill would be motivated to make such a combination because of the advantages of being able to wash away unbound probe in the solid phase system disclosed by Babon.

Applicants respectfully disagree, particularly in view of the amendments. First, the probes of Grossman differ from those of Applicants' invention in several important respects, including: (i) Grossman does not disclose capture moieties attached to probes, as pointed out by the Examiner, and (ii) Grossman discloses oligonucleotide probes only derivatized with *polymer chains* to generate distinct electrophoretic mobilities, whereas Applicants' invention employs probes with *non-oligomeric* moieties to generate distinct electrophoretic mobilities. The latter difference is important because the non-oligomeric moieties are small molecules, as pointed out on page 14, line 34, of the specification; consequently, they result in faster separations and reduced likelihood of interference with enzymatic cleavage during the assay.

Second, Grossman and Babon neither disclose nor suggest the desirability of placing a capture ligand on the probe, as described by Applicants. In this regard, Applicants direct the Examiner to page 24, line 5, to page 25, line 36, of the specification and Figs. 26 and 27 of the application which show the dramatic improvement in signal that occurs by use of capture ligands on probes. As explained in the above passage, capture ligands, such as biotin, are used with a capture agent, such as avidin, to exclude interfering material from the electropherograms, with the dramatic result exemplified by Figs. 26 and 27. The capture ligands of Applicants' are NOT used in a wash step, as disclosed in Babon or in Murtagh, Jr. et al (U.S. patent 5,744,306, see Fig. 16 and col. 31, lines 58-62), but rather are used to impart a mass and charge on uncleaved or partially cleaved probes that excludes them from the electropherogram of the cleaved eTag reporters (see the section of the application entitled, "C. Capture Ligands," cited above).

There is no equivalent observation to those of Figs. 26 and 27, or other suggestion, in either Grossman or Babon or Murtagh that would motivate one of ordinary skill to place the capture ligand of Babon or Murtagh on the probes of Grossman to achieve the results of Applicants' invention. Applicant submit that the combination of Grossman and Babon would not lead one of ordinary skill to Applicants' invention without an independent inventive contribution, and accordingly respectfully request that the rejection be withdrawn.

The Examiner rejected claim 22 under 35 U.S.C. 103(a) as being unpatentable over Grossman (cited above) in view of Babon (cited above), and further in view of Huie (5,470,967).

The Examiner applies Grossman and Babon as above and cites Huie for the disclosure of "nuclease-resistant" linkages in oligonucleotides. The Examiner argues that it would have been obvious to one of ordinary skill to introduce nuclease-resistant linkages into the probes of Grossman using the teaching of Huie.

Applicants respectfully disagree. As pointed out in the prior Amendment, neither Grossman nor Babon nor Huie teach or suggest the analytical problem created by using a nuclease to cleave a probe. A nuclease does not always cleave every probe at precisely the same inter-nucleoside linkage, as is illustrated diagrammatically in Figure 3A-C of the application. As a result, such cleavage can give rise to spurious peaks upon electrophoretic separation. There is no suggestion or appreciation of this problem in either reference. In regard to the Examiner's comment that Grossman teaches the use of the exonuclease activity of a polymerase to degrade a probe (Figs. 19A-19B), the important point is that *Grossman did not appreciate the problem* created by using such a polymerase. That is, the problem of the imprecise cleavage by the polymerase that gives rise to multiple degradation products which, in turn, gives rise to spurious peaks upon electrophoretic separation.

As pointed out in the prior Amendment, Huie is concerned with the use of nuclease resistant oligonucleotides for therapeutic purposes; thus, the application of such compounds in analytical applications is simply not disclosed or suggested, and clearly the problem of multiple degradation products causing spurious peaks on an electropherogram is neither disclosed nor suggested in either Huie or Grossman or Babon.

In view of the above, Applicants submit that one of ordinary skill would not be motivated to combine the teachings cited above to obtain Applicants' invention. Accordingly, Applicants respectfully request that the rejection be withdrawn.

In view of the above, Applicants submit that the claims as written fully satisfy the requirements of Title 35 of the U.S. Code, and respectfully request that the rejections thereunder be withdrawn and that the claims be allowed and the application quickly passed to issue.

If any additional time extensions are required, such time extensions are hereby requested. If any additional fees not submitted with this response are required, please take such fees from deposit account **50-2266**.

Respectfully submitted,



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Enclosures:

Petition for Time Extension
Declaration of Sequence Listing with CRF on 3.5 inch diskette
Facsimile Transmittal cover sheet with deposit account withdrawal
authorization.



Exhibit A

Paragraphs Added or Modified in the Specification Showing Amendments (if applicable)

Page 13, after line 2, please insert the following paragraph from parent application 09/698,846:

-- The eTag reporters will vary depending upon the method of detection. Groups of at least 10 eTag reporters bound to 10 different binding compounds will be used in the determinations. The eTag reporters will be characterized by being cleavable from the binding compound in the same vessel by the same cleavage mechanism, having a shared characteristic that permits separation and individual detection, being compatible with the determination method and being in the molecular weight range of about 30 to 3000 dal, usually in the molecular weight range of about 35 to 1500 dal. The variation may be mass using a mass spectrometer, where a magnetic field is used for separation, mass/charge ratio using electrokinesis, where an electric field is used for separation, which may also include sieving and/or adsorbing polymers, adsorption, using chromatography, e.g gas chromatography, high pressure liquid chromatography, where polar and van der Waal interactions are used for separation, etc.—

After page 67, please add the following Sequence Listing:

-- Sequence Listing

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MATRAY, Tracy
CHENNA, Ahmed
<120> Sets of oligonucleotide-binding e-tag probes
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4--

Currently Pending Claims Showing Amendments (if applicable)

16. (Amended) A set of electrophoretic probes for detecting the presence or absence of one or more of a plurality nucleotide sequences in a sample, the set comprising a plurality of electrophoretic probes selected from the group defined by the formula:

(D, M)-N-T

wherein:

(D, M)-N is an e-tag reporter released from an electrophoretic probe of the set upon digestion of the electrophoretic probe by a nuclease;

D is a detection moiety;

M is a non-oligomeric compound [~~mobility modifier~~] consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron;

N is a nucleotide; and

T is an oligonucleotide specific for at least one of the plurality of nucleotide sequences, each T having a length in the range of from 12 to 60 nucleotides such that at least one nucleotide of T has a capture ligand attached;

and wherein each e-tag reporter of the plurality of electrophoretic probes has a distinct charge/mass ratio so that e-tag reporters of different [~~the plurality of~~] electrophoretic probes form distinct peaks in an electropherogram upon electrophoretic separation;
and wherein the capture ligand specifically binds to a capture agent to exclude undigested electrophoretic probes from the electropherogram.

17. The set of claim 16 wherein said plurality is in the range of from 5 to 100 and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

18. (Cancelled) [The set of claim 17 wherein said distinct charge/mass ratio is in the range of from 0.001 to 0.5.]

19. The set of claim 17 wherein D is a fluorophore, chromophore, or an electrochemical label.

20. (Amended) The set according to claim 16, 17, [18,] or 19 wherein said formula is D-M-N-T.

21. (Amended) The set of claim 20 wherein said capture ligand is biotin and wherein said capture agent is avidin.

22. The set of claim 20 wherein said oligonucleotide has at least one nuclease-resistant linkage.

23. The set of claim 20 wherein said fluorophore is a fluorescein.

24. (Amended) The set of claim 20 wherein M consists [~~is a mobility modifier consisting~~] of from 2 to 100 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

25. (New) A set of electrophoretic probes for detecting the presence or absence of one or more of a plurality nucleotide sequences in a sample, the set comprising a plurality of electrophoretic probes selected from the group defined by the formula:

(D, M)-N-T

wherein:

(D, M)-N is an e-tag reporter released from an electrophoretic probe of the set upon digestion of the electrophoretic probe by a nuclease;

D is a detection moiety;

M is a non-oligomeric compound having a molecular weight of between 35 and 1500 daltons;

N is a nucleotide; and

T is an oligonucleotide specific for at least one of the plurality of nucleotide sequences, each T having a length in the range of from 12 to 60 nucleotides such that at least one nucleotide of T has a capture ligand attached;

and wherein each e-tag reporter of the plurality of electrophoretic probes has a distinct charge/mass ratio so that e-tag reporters of different electrophoretic probes form distinct peaks upon electrophoretic separation

and wherein the capture ligand specifically binds to a capture agent to exclude undigested electrophoretic probes from the electropherogram.

26. (New) The set of claim 25 wherein D is a fluorophore, chromophore, or an electrochemical label.

27. (New) The set according to claim 26 wherein said formula is D-M-N-T.

28. (New) The set of claim 27 wherein said capture ligand is biotin and wherein said capture agent is avidin.

29. (New) The set of claim 27 wherein said e-tag reporter is selected from the group consisting of the following compounds:

